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Resistance of Red Clovers to Sclerotinia Trifoliorum Erik., and Infection Studies.

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ADDENDUM

The fungus referred to as *Gibberella saubinetii*, on page 123 of this bulletin (Ky. Exp. Sta. Bul. 341), was assumed to be identical with the fungus *Fusarium roseum* Lk. isolated from clover by Selby and Manns (Ohio Exp. Sta. Bul. 203, 1909) and considered by them to be identical with the wheat scab organism *G. saubinetii*. While the fungus from clover leaves, and an apparently identical one isolated frequently from crowns of dying red clover plants, appear like the wheat scab organism in culture, a study of the fungus from the clover crowns, made since this bulletin was printed, shows that it is different from *G. saubinetii*, which raises the question as to whether the latter organism is as common a clover pathogen as has been supposed. Both organisms produce the deep carmine color on potato dextrose agar, and the ærial hyphæ of both sometimes turns yellowish brown, but old cultures of *G. saubinetii* are darker than those of the clover organism. The macroconidia of the clover organism are more curved and tapering than those of *G. saubinetii*. The opinion that it and the wheat-scab organism are different was confirmed by Dr. C. D. Sherbakoff, to whom a culture and drawings of the clover organism were submitted. He stated: "Morphologically, the fungus is nearest to *Fusarium trifolii* Jaczweski. It should be considered the same as *F. roseum* v. *Woronini* Naumov."

ERRATUM

Page 122. Footnote should read Bul. 339.

BULLETIN NO. 341

(Research Bulletin)

Resistance of Red Clovers to Sclerotinia Trifoliorum Erik., and Infection Studies.

W. D. VALLEAU, E. N. FERGUS, and LAWRENCE HENSON[1]

In an earlier publication Fergus (4) gave some evidence that a red clover variety (*Trifolium pratense L.*) grown for many years in Central Kentucky was more resistant to *Sclerotinia trifoliorum* Erik. than varieties obtained from other parts of North America and from Europe. Similar reports of resistance of local red clovers to *S. trifoliorum* have come from other regions. Baudys (1) reports that Hiltner in Bavaria and Zimmerman in Mecklenburg concluded from their experiments in 1911, '12 and '13, that the disease chiefly attacks clovers of foreign origin; and Ulander in Sweden stated in 1915 that local wild-growing, white clover, and late-flowering Swedish red clover are resistant. These views were in contradiction with Kutin's observations in 1913, as he writes that in that year all indigenous clovers in Bohemia suffered heavily. Esmarch (3) and Pape (10, 11) report that the disease is worse on foreign red clovers than on native varieties. Troussova (13) found red clover from the central black-soil wheat zone of Russia to have a higher incidence of disease than clover grown locally about Moscow.

Comparative Resistance of Year-Old Red Clovers, 1932. The winter of 1931-32 was exceptionally mild in Central Kentucky, and appeared to favor the development of crown rot of red clover. During the spring of 1932 the disease was prevalent thruout a series of 78 plots[2] of one-year-old clover, each

[1] Agent, Bureau of Plant Industry.

[2] Seed for these plots was furnished by Dr. E. A. Hollowell, of the Bureau of Plant Industry, for cooperative studies on adaptability of red clovers.

6 x 75 feet, growing on the Kentucky Experiment Station farm at Lexington. Every third plot was sown with an adapted variety (Ky. 101, B. P. I. 16052) giving an excellent opportunity for comparing the relative resistance of it with unadapted clovers from Europe and various parts of North America, under conditions of severe infestation. On April 6, 1932, counts were made in 1-meter squares, selecting an area which appeared to be typical of the plot. The dead, infected, and apparently healthy plants in each square were recorded. In a duplicate series, estimates of the total number of dead and injured plants were made without the estimator knowing the strain of clover concerned. These estimates corresponded very closely with the counts. A summary of the counts made in the first series is given in Table 1, with the estimated stand remaining December 4, 1932.

Observations during the spring indicated that infection was general and quite uniform over both series of plots. Further evidence of uniformity of infection was obtained in the fall when, on October 21, 1932, counts were made of apothecia of *S. trifoliorum*. A frame a foot square was thrown at random into two separate parts of each of the 78 plots and the area within the frame was examined. Apothecia were found in 150 of the 156 square-foot areas. The number per square foot, counted in 40 areas, ranged from 4 to 97, with an average of 26.4 apothecia per square foot, or 1,149,984 per acre.

The Kentucky clover (101) had an average of 71.5 percent of plants apparently unaffected, in 11 plots. In contrast, foreign clovers were very much reduced in stand, with an average of 26.6 percent of unaffected plants, in three plots (Table 1). An English strain had only 13 percent unaffected plants. Tennessee anthracnose-resistant clover was somewhat less resistant than Kentucky clover. Clovers from other parts of the United States varied in resistance from that of a Washington clover, in which 2/3 of the plants were affected, to others nearly as good as the Kentucky variety. The degree of adaptation of these clovers to the regions from which the seed was obtained was usually unknown. The evidence presented seems to leave

no doubt that spring-sown American clover varieties, when grown together at Lexington, show great differences in injury from *S. trifoliorum* when a year old, and that a relatively high degree of what appears to be resistance results from continuous selection, thru a period of years, in Central Kentucky.

TABLE 1. Summary of counts of red clover plants uninjured by S. trifoliorum, April 6, 1932, and estimated stand remaining December 4, 1932.

Source of Seed	No. of Plots.	Uninjured Plants, Percent	Range Percent	Stand Dec. 4, 1932
Kentucky (101)	11	71.5	66–79	95*
Tenn., No. Car., Va.	7	53.4	43–67	68
Ohio, Minn., Wis., Canada	4	54.7	25–63	68
Wash., Ore., Colo., Ida.	13	48.0	21–78	8
English, French, Russian	3	26.6	13–42	22

* 100 percent = 3 plants per square foot.

Injury to Two-Year-Old Adapted Clover. By the fall of 1932 the stands in the plots under discussion were all much reduced (Table 1) and were unusually heavily infected with spores from the apothecia already mentioned. During January and February, 1933, *Sclerotinia trifoliorum* became active and death of the two-year-old plants remaining in all the plots except the Kentucky 101 gradually occurred. By spring the position of the Kentucky 101 plots was clearly indicated by scattering clover plants, perhaps 1 to every square yard, while the other plots were without clover plants.

Comparative Resistance of Fall-Sown Clovers. In the fall of 1932, 9-foot rows of 11 red clovers from various sources were sowed 8 inches apart in steamed soil. During October apothecia were planted between the rows, many being placed between certain rows and very few between others. Leaf-spots were soon abundant in the immediate vicinity of the apothecia. Dead plants were not found until after several days of warm, damp weather in January. During February the disease

progressed until by the first of March practically all the plants were dead in the rows between which numerous apothecia had been placed. Where but few apothecia were placed, many plants were still alive the first of May. Adapted clover sown in the fall appeared to show no more resistance than unadapted clovers.

The plants which remained alive were surrounded by dead plants, and dead plants were scattered in the rows. Dead plant material was also washed upon them many times during late winter and spring, by the exceptionally frequent rains. Under these conditions, which appeared ideal for the saprophytic stage of the organism to bring about infection, the surviving plants remained unaffected.

In several of the rows enough hard seed remained in the soil over winter so that an excellent stand resulted soon after the majority of the overwintering plants died. The seedlings grew up thru the dead plant debris but remained unaffected by *S. trifoliorum*. This suggests that *S. trifoliorum* soon ceases vegetative activity when dependent on dead plant material for subsistence.

A second series of 50 rows of red clover consisting of 20 Kentucky clovers and 7 from northern and western states and Europe was sowed in the fall of 1932. The location was such that ascospores of *S. trifoliorum* were blown upon the clovers from the 1931-32 series. In the late fall *S. trifoliorum* leafspots were abundant. During late January and February crown rot developt but more slowly than in the nearby more heavily infected plants in steamed soil. Final records were made March 29, 1933, the results of which are given in Table 2. Kentucky clovers were less injured than unadapted clovers as a group, altho the stand of all of them was so much reduced as to have little practical significance except from the standpoint of selection. It is unusual to sow red clover in the fall in Kentucky so that all the clovers may be considered to have been growing under abnormal conditions.

Year-Old Clover. An extensive series of spring-sown red clovers, one year old, were under observation thruout the winter and spring of 1932-'33. Crown rot developt in a few

plants in February and occasional dying plants were found later. The field was so situated that infection from windblown ascospores was not likely to have been abundant.

TABLE 2. Resistance of clovers sown in the fall.

Dead Plants, Percent	Number of Rows	
	Kentucky	Unadapted
80	7	-----
85	2	-----
90	2	1
95	14	4
98	4	11
99		1
100		4

Origin of Field Infection. There appears to be no general agreement as to the origin of the infection in new sowings of clover. Studies in Europe and America have demonstrated that sclerotia may be carried with seed, and it has been suggested that they may be the direct source of infection in the field. Seed has been found to contain the fungus and this has been considered a possible means of dispersal and of field infection. Sclerotia and affected seed may have played a part in geographic distribution of the fungus in the past, but now are probably minor factors in field infection in regions where clovers and alfalfa have been grown for years.

Direct infection from wind-blown ascospores is an explanation which does not seem to have been given the consideration which it deserves by several writers, altho it appears to be a satisfactory explanation of field infection in any region where clovers or alfalfa are generally grown. The nearly perfect infection of the 1931-32 plots previously discussed cannot be attributed to soil infestation with a vegetative stage of the fungus or sclerotia, as legumes had not been grown on this field for many years; or to seed infection or direct infection from sclerotia carried in the seed, as these could hardly have been present in all lots in sufficient amounts to cause such extensive infection.

It may, however, be accounted for by wind-blown ascospores, as red clover and alfalfa had been grown on the windward side of this plot regularly for years. As ascospores are discharged over a period of about 2 months in the fall, the opportunity for infection by this means was great. In every second-year field of clover and alfalfa examined in Fayette county, the fall of 1932, apothecia were found in abundance. In clover fields sown in the spring of 1932, apothecia were rare, only a single one being found in several fields examined. Considering the relatively large numbers of apothecia which must result from only mild attacks of crown-rot, wind-blown ascospores would appear to be the most usual source of infection in new plantings.

Method of Ascospore Infection. Little exact information seems to be available as to the means by which infection results from ascospores. Wolf and Cromwell (15) state that Rehm, in 1872, "suspended mature fruit bodies over healthy plants and within 6 to 8 days the mycelium was present within leaf blades, the plants subsequently collapsed, and sclerotia were formed in due time." They also state that Coleman, in 1907, "reported that young clover plants only can be readily infected by ascospores, altho under favorable conditions older plants were in a few instances attacked." These earlier studies on direct infection from ascospores have been given little consideration by later workers, primarily because decay of the plants did not soon follow ascospore infection. Wolf and Cromwell state that because infection "begins on the stem at or near the ground level it is believed that the vegetative mycelium is responsible for the disease and that little disease results naturally by direct infection with ascospores." Gilbert and Myer (6) state that "during the winter and early spring the fungus exists in its vegetative condition, first as a saprophyte in the soil and then as a parasite upon the host plants" and that when ascospores first germinate, they send out "mycelial threads among the soil particles until these threads come in contact with young alfalfa or clover plants when infection takes place." Gilbert and Bennett (5) state that "after the first warm,

moist days in February, growing hyphae may be found on the surface of the soil in the field and soon after infection of the host plants may be observed." Wadham (12) failed to obtain infection with ascospores in pure water unless the leaves were

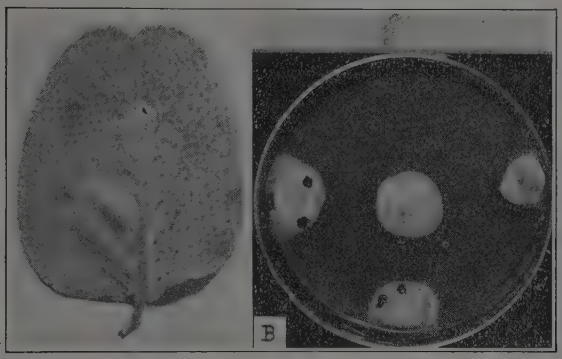

Figure 1. A. A leaflet of red clover infected with S. trifoliorum as a result of planting apothecia around fall-sown red clover plants in the field. X 2.3. Photograph December 20, 1932. B. Isolation of S. trifoliorum from spots on leaves of greenhouse-grown red clover plants which had been confined under a bell jar with mature apothecia of S. trifoliorum. Apothecia placed with plants November 4, plantings on plates made November 15, photographed December 13, 1932. The leaf tissue was dipped for 10 seconds in 1:1000 mercuric chloride and washed in water before planting.

injured or a small amount of nutrient was added. He thought that a saporphytic mycelium must first be developt before living plants can be attacked. Hino (7) suggested that "during the growing season the stem bases of the plant may be infected by mycelium arising from discharged ascospores which have germinated in the soil. The primary source of infection, however, may be the mycelium arising directly from the sclerotia in the soil or mixed with seeds."

Jaczewski (8) believed that ascospores formed the principal if not the exclusive means of infection of clover plants. He observed leafspots, due to ascospore infection, but believed, with Coleman, that these spots develop no further. When weakened or partly dead leaves were infected, the fungus spread rapidly and could go unhindered to healthy parts of the plant. He believed that leaves dying in the fall at the time of ascospore

discharge formed the principal means of access of the fungus to the crowns of the plants.

In the present investigation Henson, while making apothecial counts in the fall, noticed numerous minute spots originating usually on the under side of red clover leaves, and on petioles which were directly above or near apothecia (Figure 1, A). Isolations from the leaf and petiole spots, either with or without preliminary treatment in 1:1000 bichloride of mercury, resulted in pure cultures of *S. trifoliorum* from many of the spots cultured (Figure 1, B and Table 3). When apothecia were placed under bell jars with greenhouse-grown red clover plants, similar spots develop on leaves and petioles, from which the organism was isolated in pure culture. An examination of fall-sown clover plants which were about two hundred yards from the leeward side of the clover plots in which numerous apothecia were present, showed many of the same type of spot to be present. *S. trifoliorum* was isolated from some of these spots. Fall-sown red clover plants, in steamed soil, amongst which apothecia were planted, develop an abundance of the leaf spots, and from some of them *S. trifoliorum* was isolated after surface sterilization of the leaves. Affected leaves were still abundant in this planting on December 30 after a period of low temperature and snow. The spots were small and were frequently only on one half of a leaflet (Figure 1, A). This is because the leaflets, when young, are folded at the midvein and only the lower half is exposed directly to the discharging spores. After the leaves unfold infection also occurs thru the upper leaf surface and is then not visible on the under surface for several days. Both old and young leaves of plants exposed to apothecia in the greenhouse became infected thru uninjured surfaces. The leaf spots increase in size very slowly, both affected and unaffected leaves of plants in the field dying from other causes during the winter. A new crop of leaves gradually develops as weather conditions permit, and these, in turn, may be affected by a similar but distinct spot, apparently caused by *Phoma trifolii* J. and V.* In early January, *S. trifoliorum* was still present in the

*Johnson, E. M. and W. D. Valleau. Black-stem of alfalfa, red clover and sweet clover. Ky. Agr. Expt. Sta., Bul. 340, 1933.

TABLE 3. Isolations of S. trifoliorum from leafspots, petiole spots and decaying petioles, fall and winter of 1932-33. Greenhouse and steamed-bed plants were inoculated by planting mature apothecia near them on the ground.

Date.	Material.	Treatment.	Total Plantings.	Total S. trifoliorum
Nov. 1	Leafspots, G. H.*	Sterile water	55	55
Nov. 10	Leafspots, G. H.	$HgCl_2$ 30 seconds**	20	8
Nov. 12	Petiole spots, G. H.	$HgCl_2$ 90 seconds	10	3
Nov. 12	Leafspots, G. H.	$HgCl_2$ 90 seconds	15	15
Nov. 15	Leafspots, G. H.	$HgCl_2$ 10 seconds	70	50
Dec. 18	Decaying petioles, G. H.	Sterile water	10	10
Dec. 30	Leafspots, G. H.	Sterile water	40	12
Nov. 3	Leafspots, steamed bed	$HgCl_2$ 10 seconds	8	4
Nov. 11	Leafspots, steamed bed	$HgCl_2$ 90 seconds	50	2
Dec. 6	Leafspots, steamed bed	$HgCl_2$ 10 seconds	35	21
Dec. 30	Leafspots, steamed bed	$HgCl_2$ 20 seconds	60	0
Dec. 30	Leafspots, steamed bed	$HgCl_2$ 10 seconds	50	14
Jan. 14	Leafspots, steamed bed	Sterile water	65	1
Jan. 30	Decaying petiole, steamed bed	$HgCl_2$ 10 seconds	40	2
Feb. 7	Decaying petiole, steamed bed	Sterile water	70	14
Nov. 3	Leafspots, field	$HgCl_2$ 10 seconds	8	2
Dec. 6	Leafspots, field	$HgCl_2$ 10 seconds	35	8
Dec. 6	Leafspots, 2nd year clover field	$HgCl_2$ 10 seconds	35	14

* Plants in greenhouse.
** Treated in 1:1000 mercuric chloride and washed in 6 changes of sterile water.

leaf spots of green leaves, altho the spots had increased in size only slightly. At that time, isolation of *S. trifoliorum* was less frequent than earlier, many of the spots yielding *Gibberella saubinetii*. The failure of numerous leaf and petiole spots to cause decay of the fall leaves of either adapted or unadapted clovers indicates a high degree of resistance of the leaves to *S. trifoliorum* at that time. Following death of the leaves *S. trifoliorum* spreads in the leaf tissue and has been demonstrated to be present in some of the decaying petioles, thru which it has

ready access to the crown (Table 3). A saprophytic stage of the fungus has been observed to develop from affected leaves which have dropped to the ground and died. This appears to be the origin of the saprophytic stage described by others, but usually considered to result directly from ascospores.

It does not seem probable that the wefts of the fungus frequently found on the ground have resulted from ascospore germination on debris as is commonly claimed, as they do not develop in the fall, but are found after the majority of affected leaves have died, and crown rot is developing. These wefts have been seen in the greenhouse where spotted leaves have died and then drooped to the ground, or where a group of dying leaves have made a larger mass of material on which the fungus can grow. The evidence is not convincing that this fungus can withstand competition with other microorganisms in previously decayed plant material, or in the soil or that it will live on previously decayed material. Production of sclerotia, as a means of carrying the fungus over unfavorable periods, suggests that the fungus in its vegetative form is not a soil habitant at all.

The evidence presented shows that *S. trifoliorum* may remain alive in leafspots for several months or as long as the leaves are alive; and that, with the death of leaves due to natural causes, the fungus spreads into the petioles and thence into the crowns. It is evident, therefore, that infection of green leaves, believed by Coleman and Jaczewski to be of no importance in the life history of the fungus is, in fact, the principal means by which the fungus is carried over from the time of spore discharge until weather conditions and the susceptibility of the plant are such that crown rot can develop. It is also evident that a saprophytic stage of the fungus, if it occurs, is secondary to the parasitic stage.

The Saprophytic Stage of Sclerotinia trifoliorum. Several writers have claimed that *S. trifoliorum* develops a saprophytic stage directly with the germination of the ascospores and that this is the chief means of existence of the fungus from the time of spore discharge until rot occurs. The evidence presented in this paper seems sufficient to exclude this phase of a

saprophytic stage from further consideration in the life history of the fungus.

A saprophytic stage, if it can be so called, develops with the death of the affected leaves, which releases the fungus from leafspots. Its activity seems to increase as plants are killed by the fungus because of a larger amount of material on which to subsist. It is possible that if favorable weather conditions persist following the first onset of the disease during the winter or spring, when the majority of the primary inoculum is released from the leafspots, infection may spread somewhat from plant to plant but this has not been demonstrated. A 9-foot square plot of *Trifolium subterraneum* L., fall sown, which completely covered the ground with a luxuriant growth, was located on a slight slope just below the red clovers, in steamed soil, thru which apothecia were scattered. In January scattered plants of the subterranean clover developt crown rot. The affected areas gradually increased in size, some of them coalescing, leaving a thick mat of decayed tissue extending irregularly thruout the plot. It appeared that all the plants would be destroyed in a short time. Decay stopped early in March, altho there was much rain in March and April which seemed to afford ideal conditions for spread of crown rot. In May the entire plot was again nearly overgrown with this clover and but little sign of the earlier attack of Sclerotinia rot was evident. Seedlings growing up thru a mat of plants recently destroyed by *S. trifoliorum*, which failed to develop the disease, have been mentioned. These and other observations of a similar nature raise a question as to the importance of the part which the saprophytic stage plays in the field and seem to emphasize the part which direct infection plays.

Possible Nature of Resistance to Sclerotinia trifoliorum. Adapted varieties of red clover, when spring sown, are undoubtedly less injured by *S. trifoliorum* than unadapted American and foreign clovers. When fall sown, the adapted clovers seemed to survive slightly better than the unadapted, but the difference was slight (Table 2.) It seems from these results that adapted clovers do not differ genetically from the

unadapted as far as inherent resistance to *S. trifoliorum* is concerned. It is also probable that physiological forms of the fungus are not concerned in the problem even tho they exist. Nilsson-Leissner (9) presented evidence that physiological forms of *S. trifoliorum* exist which appear to affect different species of clover to different degrees. It seems that we must look to the physiological condition of the plants themselves for an explanation of the differences shown between varieties in degree of injury in the spring of 1932.

It seems that local red clovers, because of a higher degree of adaptation to all environmental conditions, function in a more normal manner thruout the summer, winter and spring and consequently remain in a more resistant condition than varieties unadapted to Kentucky conditions. Thus the pathogenicity of the fungus may be much the same the world over, but in each particular region the local clovers may be most resistant because they make the most nearly normal growth. If this organism is similar in its method of penetration of the host tissue to *S. fructicola* (Wint.) Rhem, and it appears to be, then relative solubility of the middle lamella, or the outer cell wall layers, would determine susceptibility (14). It is now well known that growing conditions affect the structure of cell walls (2) and consequently the susceptibility of the tissue to penetration by certain types of pathogens.

Adaptation to Central Kentucky conditions or to the conditions in any other region where local clovers are resistant to *S. trifoliorum* would include resistance to leaf-spot organisms, petiole pathogens, rootlet rotting organisms, insect attack (leaf hopper), and perhaps other factors which would affect food storage or the level of reserves stored in the plants when going into winter. An adapted clover probably uses reserves more economically, especially during mild winters, than unadapted clovers, thus leaving larger reserves for rapid, normal spring growth. For example, the roots of 10-month-old French clover plants were found to be practically depleted of starch reserves by the middle of February, 1933, while starch was abundant in roots of adapted clover.

Direct ascospore infection of clover leaves occurs during the fall; but despite heavy infection, crown rot does not develop until months later, when the leaves present in the fall have died, and new growth has been made during a period of relatively low temperature. The newly developt tissue appears to be more susceptible than that produced during late summer and fall. Susceptibility is not due to the juvenile tissue itself, as young leaflets just unfolding in the fall, appear to be as highly resistant to spread of *S. trifoliorum* as older leaves.

If susceptibility in winter is due to the type of tissue laid down during this period, then it is not difficult to understand why adapted clover, well supplied with food reserves and adapted over a period of years to Kentucky winters, might lay down a type of tissue more resistant to *S. trifoliorum*. The theory of fluctuating physiological resistance rather than permanent genetic resistance gives a plausible explanation for a relatively high mortality of adapted clovers during unusually warm winters, as in the case reported by Kutin (1) and in the fairly high mortality (nearly 20 percent) of the Kentucky adapted variety 101 in 1932 and the nearly complete failure of all fall-sown clovers in 1933 if heavily infected. If this is the proper explanation of resistance, then varieties resistant to *S. trifoliorum* in one region cannot be expected to have equal resistance in other regions. The observations and tests here reported seem to show that clover tissue produced during periods of high temperature are likely to be highly resistant to *S. trifoliorum* while tissues produced during periods of low temperature are likely to be more susceptible, especially if the clover variety is an unadapted one.

Spring Apothecia. The majority of apothecia of *S. trifoliorum* are produced in the fall and, for the most part, winter and spring attacks of the fungus can be correlated with this fact. However, in the spring of 1933, nearly two months after the major attack of the disease in the field, isolated plants were found dying from *S. trifoliorum* which could hardly be attributed to fall infection. A single apothecium was found April 17, 1933, in a field of wheat which had recently been in

legumes and on May 5, 1933, and later, many apothecia were found in another field. This field was sowed with a mixture of legumes the winter of 1930-31 and sclerotinia rot was destructive. In 1931 and 1932 it was planted with potatoes. The winter of 1932-33 it was again sowed with a legume mixture, principally alfalfa. Sclerotinia rot killed many of the plants during late winter and spring. On May 10 apothecia were examined in order to determine whether they had originated from old sclerotia or from sclerotia produced in 1933. The apothecia were nearly all in close contact with the ground. Forty-six were dug and examined. Eight of these, from which the apothecia arose, were on the surface but only two were associated with dead plant material. The remainder were buried from ¼ to 1½ inches below the surface (Table 4) and were unassociated with recently killed plants.

TABLE 4. Depth to which sclerotia were buried from which spring apothecia were produced. Examination May 10, 1933.

Depth in Inches						
On surface	¼	½	¾	1	1¼	1½
8	7	11	8	9	2	1

Five hundred sclerotia were collected from crowns of recently killed plants. These showed no evidence of apothecial production. Examinations of 4 plots, 3 of which were in steamed soil, of fall-sown clover injured by sclerotinia rot in January and February, 1933, revealed numerous sclerotia but no apothecia on May 10. There is little doubt that the spring apothecia originated from sclerotia approximately 2 years old.

Ascospore cultures were obtained from the spring apothecia, which appear identical with cultures obtained the previous fall from leafspots resulting from ascospore infection. Apothecia placed on the ground beside red clover plants soon produced leafspots of the type shown in figure 1, and *S. trifoliorum* was isolated from some of the spots. It is thus probable that a part of the spring infection in Kentucky results from apothecia produced in the spring.

Additional Hosts of Sclerotinia trifoliorum. Dead plants of *Plantago lanceolata* L. were found among rotting clover plants. Numerous sclerotia typical of the clover fungus were found buried in the crowns. In a field of crimson clover, plants of *Lactuca* sp. growing in an area affected by *S. trifoliorum* were dying and *S. trifoliorum* was isolated from them. Pyrethrum (*Chrysanthemum cinerariaefolium* Vis.) which was being tested on the Station farm was found by R. E. Culbertson to be dying from a crown rot the spring after they were set. A species of Sclerotinia was isolated from them and in extensive comparative studies with the clover organism was found to be identical with it. At the suggestion of the senior author, Culbertson made a detailed comparative study of the published literature on *Sclerotinia minor* Jagger and *S. trifoliorum* and was unable to find any real difference between the two organisms. It appears, therefore, highly probable that *S. minor* is merely *S. trifoliorum* occurring on a host, usually lettuce, not commonly recognized as a host of *S. trifoliorum*.

SUMMARY

1. Red clover varieties adapted to Central Kentucky conditions are less injured by stem rot caused by *Sclerotinia trifoliorum* Erik., if spring sown, than other American and European varieties of red clover.

2. Resistance appears to result directly from a higher degree of adaptation to local environmental conditions, which in turn results in tissues of a higher degree of resistance, rather than resistance to local physiological forms of *S. trifoliorum* or to an inherent genetic resistance.

3. Infection of new plantings of clover and alfalfa appears to result directly from wind-borne ascospores and not from soil infestation, sclerotia introduced with seed, or hyphae in seeds.

4. Infection of leaves and petioles of red clover occurs directly from germinating ascospores. Following death of the leaves, due to other causes, *S. trifoliorum* is found in the dying petioles and may thus have direct access to the crown of the plant. The saprophytic stage of the fungus appears to be sec-

ondary to the parasite stage in the leaf and of little importance except perhaps when plants are dying rapidly from direct infection.

5. *Plantago lanceolata* L., *Chrysanthemum cinerariaefolium* Vis., and *Lactuca* sp. were found to be hosts of *S. trifoliorum*. The suggestion is made that *S. minor* Jagger and *S. trifoliorum* are identical.

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